

**Amendments to the Claims:**

This listing of claims will replace all prior versions and listings of claims in the application.

**Listing of Claims:**

1-24. (Cancelled).

25 (Currently Amended). An induction solution for rapid detection of coliforms, capable of inducing the expression of inducible enzymes  $\beta$ -glucuronidase and  $\beta$ -galactosidase, in the absence of cell growth, said solution comprising:

at least one amino acid or a mixture of amino acids being at a concentration that does not in such a quantity to not allow a detectable coliform growth within a time period of, between 0 and 120 minutes when the coliform cells come in contact with said at least one amino acid or said mixture of amino acids and wherein said amino acid concentration is at about, a detectable cell growth of coliforms in contact therewith 80 mM;

a buffer system;

a bivalent ion; and

an enzyme inducer consisting of isopropyl- $\beta$ -thiogalactopyranoside and/or methyl- $\beta$ -D-glucuronide,

wherein said mixture of amino acids are at a concentration up to 80mM, and

wherein said induction solution detects said coliforms in the absence of cell growth.

26. (Currently Amended) The induction solution of claim 25, wherein said at least one amino acid or said mixture of amino acids is selected from the group consisting of tryptophan (W), methionine (M), threonine (T), isoleucine (I); and leucine (L).

27. (Cancelled).

28. (Previously Added) The induction solution of claim 25, wherein said mixture of amino acids comprises alanine (A), cysteine (C), aspartic acid (D), glutamic acid (E), phenylalanine (F), glycine (G), histidine (H), isoleucine (I), lysine (K), leucine (L), methionine

(M), asparagine (N), proline (P), glutamine (Q), arginine (R), serine (S), threonine (T), valine (V), tryptophan (W), and tyrosine (Y).

29. (Previously Added) The induction solution of claim 25, wherein the bivalent ion is Mg<sup>++</sup> and said Mg<sup>++</sup> is used at a concentration of about 0.5 mM.

30. (Previously Added) The induction solution of claim 25, wherein said isopropyl- $\beta$ -D-thiogalactopyranoside is used at a concentration of about 0.2 mM and/or said methyl- $\beta$ -D-glucuronide is used at a concentration of about 2 mM.

31. (Previously Added) The induction solution of claim 25, wherein said induction solution further comprises a selective agent that acts as a membrane permeabilizer.

32 (Currently Amended). The induction solution of claim 25, wherein said selective agent is induction solution further comprises sodium dodecyl sulphate as a selective agent.

33. (Withdrawn) A method for the rapid detection of coliform bacteria, comprising:

(a) contacting a sample containing coliform cells with an induction solution according to claim 25 to form a mixture;

(b) incubating said mixture at a temperature range between 30 and 50° C;

(c) adding a fluorogenic substrate to said mixture;

(d) rupturing said coliform cells in said mixture with a lysis agent to release an induced enzyme;

(e) further incubating said mixture at a temperature of less than 50° C, for a period of time depending on cell number, to assist in the hydrolysis of said fluorogenic substrate, with discharge of the corresponding fluorophore; and

(f) raising the pH of said mixture to a range from about 11 to 12 with a basic solution of sodium hydroxide (NaOH) in water.

34. (Withdrawn) The method of claim 33, wherein said coliform bacteria is selected from the group consisting of total coliforms, faecal coliforms and *E. coli*.

35. (Withdrawn) The method of claim 34, wherein said total coliforms are detected at a temperature range of about 30°C and about 40°C and said faecal coliforms or *E. coli* are detected at a temperature range of about 30°C and 50°C.

Amendment dated July 28, 2009

Reply to Office Action of April 29, 2009

36. (Withdrawn) The method of claim 33, wherein the concentration of the corresponding fluorophore is determined by a fluorimetric assay upon extracellular hydrolysis reaction of targeted induced enzymes with said relative fluorogenic substrates selected from the group consisting of methylumbelliferyl- $\beta$ -D-galactoside for  $\beta$ -galactosidase and methylumbelliferyl- $\beta$ -D-glucuronide for  $\beta$ -glucuronidase.

37. (Withdrawn) The method of claim 33, wherein said lysis agent is chloroform and/or triton-x.

38. (Withdrawn) The method of claim 33, wherein said fluorescent substrate is selected from the group consisting of methylumbelliferyl- $\beta$ -D-galactoside in dimethylsulfoxide and methylumbelliferyl- $\beta$ -D-glucuronide in water/triton-X and relative mixtures thereof.

39. (Withdrawn) The method of claim 33, wherein said sample to be analyzed is pre-extracted with a physiological saline or a phosphate buffered saline solution.

40. (Withdrawn) The method of claim 33, wherein said sample is sterilized by passage through a 0.45  $\mu$ m or less pore size membrane filter and said filter is immersed in said induction solution.

41. (Withdrawn) The method of claim 36, wherein said fluorimetric assay is performed using an excitation wavelength that ranges from about 330 to 390 nm and an emission wavelength that ranges from about 410 to 470 nm with a slit width that ranges from about 2.5 to 20 nm.

42. (Withdrawn) An analysis kit for the rapid detection of coliform cells comprising:  
an induction solution of claim 33;  
a solution comprising a fluorescent substrate; and  
an organic solvent capable of inducing cell lysis.

43. (Withdrawn) The analysis kit of claim 42, wherein said coliform cells are selected from the group consisting of total coliforms, faecal coliforms and *E. coli*.

44. (Withdrawn) The analysis kit of claim 42, wherein said induction solution is sterilized by passage through 0.45  $\mu$ m or less pore size membrane filter and/or is in a lyophilized form.

Amendment dated July 28, 2009

Reply to Office Action of April 29, 2009

45. (Withdrawn) The analysis kit of claim 42, wherein said fluorescent substrate is selected from the group consisting of methylumbelliferyl- $\beta$ -D-galactoside, methylumbelliferyl- $\beta$ -D-glucuronide and relative mixtures thereof.

46. (Withdrawn) The analysis kit of claim 42, further comprising a fluorescence amplifier that is constituted from a solution of sodium hydroxide (NaOH) in water or any other base capable of increasing the pH range of the induction solution to about 11-12.

47. (Withdrawn) The analysis kit of claim 42, wherein said an organic solvent is chloroform and/or triton-x.

48. (Withdrawn) The analysis kit of claim 42, wherein said sample is from waste water, surface water, bathing water, freshwater, seawater, groundwater, food extracts or soil extracts.